PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 31/57 // (A61K 31/57, 31:34, 31:26, 31:21, 31:195)

A1

(11) International Publication Number: WO 95/02408

(43) International Publication Date: 26 January 1995 (26.01.95)

(21) International Application Number: PCT/EP94/02368

(22) International Filing Date: 18 July 1994 (18.07.94)

(30) Priority Data:
08/092,426 16 July 1993 (16.07.93) US

(71) Applicant: SCHERING AKTIENGESELLSCHAFT [DE/DE]; Müllerstrasse 178, D-13353 Berlin (DE).

(71)(72) Applicants and Inventors: GARFIELD, Robert, E. [US/US]; 1814 Winding Way, Friendswood, TX 77546 (US). YALLAMPALLI, Chandra [IN/US]; 1222 Berkely Lake, Houston, TX 77062 (US).

(72) Inventors: CHWALISZ, Kristof; Lobber Steig 7a, D-13342 Berlin (DE). BUKOWSKI, Radoslaw; c/o K. Chwalisz, Lobber Steig 71, D-13342 Berlin (DE).

(81) Designated States: AU, BR, CA, CN, CZ, FI, HU, JP, KR, LT, LV, NO, NZ, PL, RU, SI, SK, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: COMBINATION OF A PROGESTATIONAL AGENT AND A NITRIC OXIDE SYNTHASE SUBSTRATE AND/OR DONOR FOR THE TREATMENT OF PREECLAMPSIA AND PRETERM LABOR

(57) Abstract

Preeclampsia and preterm labor in a pregnant female mammal are treated by administering thereto a combination of a progestin and a nitric oxide synthase substrate, a nitric oxide donor or both, optionally in further combination with one or more of a cyclooxygenase inhibitor, a PGI_2-mimetic, a thromboxane (TXA_2) inhibitor, a compound possessing TXA_2-agonistic and TXA_2-inhibiting properties, a compound possessing TXA_2-antagonistic and PGI_2-memetic activities, and a TXA_2 antagonist.

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Combination of a progestational agent and a nitric oxide synthase substrate and/or donor for the treatment of preeclampsia and preterm labor.

Background of the Invention

This invention relates to a method for the treatment of preeclampsia and of preterm labor with the combination of a progestational agent and a nitric oxide synthase substrate, a nitric oxide donor or both, alone or in further combination with one or more of a cyclooxygenase inhibitor, a PGI_2-mimetic, a thromboxane (TXA_2) inhibitor, A compound possessing TXA_2-agonistic and TXA_2-inhibiting properties, a compound possessing TXA_2 antagonistic and PGI_2-memetic activities, and a TXA_2 antagonist, and to pharmaceutical compositions comprising such a combination.

Preeclampsia, toxemia or eclampsia of pregnancy can be a significant health problem during pregnancy and they are the leading causes of fetal growth retardation, fetal mortality and morbidity, premature birth and maternal mortality. The etiology of the disease is largely unknown and effective therapy is not available. Preeclampsia of pregnancy is characterized by a triad of hypertension, pathological edema and proteinuria. This disease affects 6 to 10% of all pregnancies.

Recently, nitric oxide has been shown to be endothelium derived relaxing factor (EDRF) from the endothelium of blood vessels. Nitric oxide is considered to be a major mediator in the control of vascular reactivity. Nitric oxide is synthesized from L-arginine by nitric oxide synthase located in endothelial cells. Nitric Oxide can also be generated by application of various nitric oxide donors such as sodium nitroprusside, nitroglycerin, glyceryl trinitrite, SIN-1, isosorbid mononitrite, isosorbid dinitrite, etc.

Treatment of pregnant rats with nitric oxide synthase inhibitors, which are analogues of L-arginine (such as L-NAME, N^G- nitro-L-arginine methyl ester) results in

elevated blood pressure, fetal retarded growth and proteinuria. Thus, inhibition of nitric oxide synthesis produces conditions and symptoms identical to preeclampsia of pregnancy and establishes that preeclampsia is the direct result of the decrease in nitric oxide synthesis and/or a change in the regulation of vascular tone. These conditions give rise to increased blood pressure, decreased blood flow to the fetus, retarded fetal development and proteinuria. Agents which raise nitric oxide levels therefore are useful in the treatment of preeclampsia of pregnancy. Since nitric oxide donors also reduce contractility of the uterus during pregnancy, nitric oxide donors are also useful for use in preterm labor.

The nitric oxide effects on smooth muscle depend upon the activation of guanylate cyclase and generation of cGMP to produce relaxation and this step is progesterone dependent. Thus, combinations of nitric oxide donors with progesterone are particularly efficacious for the treatment of preeclampsia and of preterm labor.

EP 0 441 119 A2 discloses the use of L-arginine in the treatment of hypertension and other vascular disorders. It suggests that the mechanism by which L-arginine is effective for this purpose is because it may be the physiological precursor of "the most powerful endothelial-derived releasing factor, nitric oxide." The use of L-arginine in combination with other pharmaceutically active agents is not discussed in this publication.

Objects of the Invention

It is an object of the invention to provide a method for the prevention and treatment of preeclampsia with a combination of a progestational agent and a nitric oxide substrate and/or donor.

It is another object to provide such a method in which a progestational agent is used in combination with

a nitric oxide substrate and/or donor for the prevention and treatment of preeclampsia.

It is a further object to provide a method for the prevention and treatment of preterm labor using a progestational agent in combination with a nitric oxide substrate and/or donor.

A further object is the provision of pharmaceutical compositions useful in practicing the methods of this invention.

Other objects will be apparent to those skilled in the art to which this invention pertains.

Summary of the Invention

In a method aspect, this invention relates to a method of treating at least one of preeclampsia and preterm labor in a pregnant female which comprises administering to a pregnant female manifesting the symptoms thereof, (a) a progestational agent and (b) one or both of a nitric oxide synthase substrate and a nitric oxide donor, alone or in further combination with one or more of a cyclooxygenase inhibitor, a PGI_2-mimetic, a thromboxane (TXA_2) inhibitor, a compound possessing TXA_2-agonistic and TXA_2-inhibiting properties, a compound possessing TXA_2-antagonistic and PGI_2-memetic activities, and a TXA_2 antagonist, in amounts effective to ameliorate the symptoms thereof, the amount of the progestational agent administered being bioequivalent to 50-300 mg. of injected progesterone and the amount of the nitric oxide synthase substrate, nitric oxide donor or both being effective to, respectively, either raise the blood level of circulating L-arginine in a pregnant female to whom the composition is administered to at least about 1 mmole above the normally 2 to 3 mmolar circulating levels or raise nitric oxide donor levels to about 1 to 100 nmolar (nanamolar).

In another method aspect, this invention relates to a method of treating preterm labor in a pregnant female which comprises administering to a pregnant female

manifesting the symptoms thereof, amounts of (a) a progestational agent and (b) at least one of a nitric oxide synthase substrate and a nitric oxide donor effective to terminate the preterm labor, alone or in further combination with one or more of a cyclooxygenase inhibitor, a PGI_2-mimetic, a thromboxane (TXA_2) inhibitor, a compound possessing TXA_2-agonistic and TXA_2-inhibiting properties, a compound possessing TXA_2-antagonistic and PGl_2-memetic activities, and a TXA_2 antagonist, the amount of the progestational agent administered being bioequiva-lent to 50-300 mg. of injected progesterone and the amount of the nitric oxide synthase substrate, nitric oxide donor or both being effective to, respectively, either raise the blood level of circulating L-arginine in a pregnant female to whom the composition is administered to at least about 1 mmole above the normally 2 to 3 mmolar circulating levels, or raise nitric oxide donor levels to about 1 to 100 nmolar.

In a product aspect, this invention relates to a pharmaceutical composition comprising (a) a progestational agent and (b) at least one of a nitric oxide synthase substrate and a nitric oxide donor, alone or in further combination with one or more of a cyclooxygenase inhibitor, a PGI_2-mimetic, a thromboxane (TXA_2) inhibitor, a compound possessing TXA_2-agonistic and TXA_2-inhibiting properties, a compound possessing TXA_2-antagonistic and PGl_2-memetic activities, and a TXA_2 antagonist, with the amount of the progestational agent per unit dosage being bioequivalent to 50-300 mg. of injected progesterone and the amount of the nitric oxide synthase substrate, a nitric oxide donor or both per unit dosage being effective to, repsectively, either raise the blood level of circulating L-arginine to at least about 1 mmole above the normally 2 to 3 mmolar circulating levels or raise the nitric oxide donor levels to about 1 to 1000 nmolar.

In another aspect this invention relates to the use of (a) a progestin and (b) a nitric oxide synthase substrate, a nitric oxide donor or both, optionally, in further combination with one or more of a cyclooxygenase inhibitor, a PGI_2-mimetic, a thromboxane (TXA_2) inhibitor, a compound possessing TXA_2-agonistic and TXA_2-inhibiting properties, a compound possessing TXA_2-antagonistic and PGI_2-mimetic activities, and a TXA_2 antagonist, for manufacture of a medicament for treating at least one of preeclampsia and preterm labor in a pregnant female mammal.

In a preferred embodiment, (a) is used in an amount which is bioequivalent to 50-300 mg of injected progesterone and (b) in an amount which is effective to raise the blood level of circulating L-arginine to at least about 1 mmole above the normally 2 to 3 mmolar circulating levels.

In a further embodiment of the invention the female mammal is a human suffering from preeclampsia.

In still a further embodiment of the invention the female mammal is a human who has exhibited or is a candidate for preterm labor.

According to a further aspect the female mammal is a human and (b) is a nitric oxide synthase substrate.

In a preferred embodiment the substrate is L-arginine.

According to another embodiment the female mammal is a human and (b) is a nitric oxide donor.

In a preferred embodiment the nitric oxide donor is sodium nitroprusside, nitroglycerin, glyceryltrinitrite, SIN-1, isosorbidmononitrite or isosorbiddinitrite.

In a further embodiment the nitric oxide donor can be for oral administration.

In another embodiment the female mammal is a human and the nitric oxide substrate or donor is for administration in combination with a cyclooxygenase inhibitor.

The use of aspirin as an inhibitor is preferred.

In still another embodiment of the invention the female mammal is a human and the nitric oxide substrate or donor is for administration in combination with a PGI_2-mimetic.

The PGI_2-mimetic is preferrably iloprost or cicaprost.

In another preferred embodiment the female mammal is a human and the progestin is progesterone.

Detailed Disclosure

The methods of this invention treat one or more of preeclampsia and preterm labor in a pregnant female mammal, preferably a human, who is manifesting the symptoms thereof or who is a high risk candidate for doing so, e.g., as determined by the progress of a present or previous pregnancy.

Because these abnormal conditions of pregnancy are produced by or aggravated by subnormal nitric oxide synthesis, both nitric oxide synthase substrates, e.g., L-arginine, and nitric oxide donors, e.g., sodium nitroprusside, nitroglycerin, glyceryl trinitrate, SIN-1, isosobid mononitrate and isosorbid dinitrate, are useful for ameliorating the symptoms thereof and, in one aspect of the method of this invention, a combination of both are employed.

A synergistic effect is achieved when a progestational agent is administered concurrently with the nitric oxide substrate and/or nitric acid donor.

Thus, the method aspect of this invention and the pharmaceutical composition aspect of this invention employs a combination of (a) a progestational agent, e.g., progesterone, and (b) either or both of a nitric oxide donor and a nitric oxide synthase substrate and, optionally, (c) one or more of a cyclooxygenase inhibitor, e.g., aspirin; a PGI_2-mimetic, e.g., iloprost and cicaprost; a thromboxane (TXA_2) inhibitor, e.g., dazoxiben hydrochloride (benzoic acid, 4-[2-(1H-imadazol1-yl)-ethoxy]-, monohydrochloride; UK 37248), dazmegrel (1H-indole-1-propanoic acid, 3-(1H-imidazol-1-ylmethyl)-2-methyl-; UK 3885), ozagrel (2-propenoic acid, 3-[4-(1-H-imidazol-1-ylmethyl)phenyl]-; OKY-046) and pirmagrel (imidazo[1,5-a]pryidine-5-hexanoic acid; CGS-13080); a compound possessing TXA_2-agonistic and TXA_2-inhibiting properties, e.g., ridogrel (pentanoic acid, 5-[[[3-pyridinyl[3-(trifluoromethyl)phenyl]methylene]-amino]oxy]-; R-68070) and labogrel (6-heptenoic acid, 7-phenyl-7-(3-

pydridinyl)-; a compound possessing TXA_2-antagonistic and PGI_2-memetic activities, e.g., 5-heptenoic acid, 7-[3-[[(diphenylmethoxy)-imino]-bicyclo,[2.2.1]hept-2-yl]-; EP 035-rac) and 5-heptenoic acid, 7-[3-[[(diphenylmethoxy)-imino]methyl]biclo[2.2.2]-oct-5-en-2-yl]- (EP 157); and a TXA_2 antagonist, e.g., 5-heptenoic acid, 7-[3-[[2-(phenylamino)carbonyl]-hydrazino]methyl]7-oxabicyclo[2.2.1]hept-2-yl]-, 1S[1.alpha.,2.alpha.(Z),3.alpha.,4.alpha.]]- (SQ 29548); benzenepropanoic acid, 2-[[3-4[(pentylamino)carbonyl]-2-oxazolyl]-7-oxabicyclo[2.2.1]hept-2-ylmethyl}-(BMS 180291); acetic acid, [4-[2-[(phenylsulfonyl)amino]-ethyl]penoxy]- (sultroban, BM-13177); benzeneacetic acid, 4-[2-[[[4-chlorophenyl)sulfonyl]amino]ethyl]- (daltroban, BM-13505); (S-145 rac); 5-hexenoic acid, 6-[3-[[[(4-bromophenyl)sulfonyl]amino]methyl]bicyclo[2.2.1]hep-2-yl]-, decyl ester, [IS[1.alpha.2.alpha.2.alpha.(Z),-3.beta.,4.alpha.]]- (ONO 8809); 9H-carbazole-9-propanoic acid, 3-[[(4-fluorophenyl)sulfonyl]amino]-1,2,3,4-tetra-hydro-, (R)- (bay-u-3405); and (4Z)-6-[(5S)-5-(4-chlor-phenylsulfonyl(aminomethyl)-cycloent-1-enyl]4-hexenoic acid (ZU 154343).

Examples of combinations of active agents which can be administered concurrently with a nitric oxide substrate and/or a nitric oxide donor and a progesterone (or other progestational agent) are low dose (e.g.,10-100 mg) of aspirin (or other cyclooxygenase inhibitor; PGI_2-mimetics (e.g., iloprost, cicaprost); combinations of a PGI_2-mimetic and low dose aspirin.

Examples of dosage ranges of typical NO-substrates and NO-donors (per os) are:

	total dose:
L-Arginine	500 mg - 10 g p.o.
Sodium Nitroprusside	range 500-2000 ug/kg/day
Nitroglycerin	0.5-10 mg
Isosorbid mononitrate	10-100 mg
Isosorbid dinitrate	10-100 mg

The following are typical oral dosage ranges active agents of the progestin and the optional other active agents concurrently administered with the nitric oxide substrate or donor:

Progestins: A daily dose bioequivalent to 50-300 mg of progesterone/day, e.g., an injectable suspension of medroxyprogersterone acetate to provide a weekly dose of thereof of 100-1000 mg or tablets or dragees providing an oral dose thereof of 5-10 mg/day; an injectable solution of hydroxyprogesterone caproate which provides a weekly dose of 250-500 mg; tablets, capsules or dragees of northindrone acetate which provide a daily dose of 5-20 mg.

Cicaprost: 5-100 ug/kg/day p.o.

Aspirin: 10-100 mg/kg/day p.o.

The pharmacologically active agents employed in this invention can be administered in admixture with conventional excipients, i.e., pharmaceutically acceptable liquid, semi-liquid or solid organic or inorganic carriers suitable, e.g., for parental or enteral application and which do not deleteriously react with the active compound in admixture therewith. Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions, alcohols, vegetable oils, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxy methylcellulose, polyvinyl pyrrolidone, etc. The pharmaceutical preparations can be sterilized and if desired mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds.

For parental application, particularly suitable are solutions, preferably oily or aqueous solutions, as well as suspensions, emulsions, or implants, including suppositories. Ampoules are convenient unit dosages.

In a preferred aspect, the composition of this invention is adapted for ingestion.

For enteral application, particularly suitable are unit dosage forms, e.g., tablets, dragees or capsules having talc and/or a carbohydrate carrier or binder or the like, the carrier preferably being lactose and/or corn starch and/or potato starch; particulate solids, e.g., granules; and liquids and semi-liquids, e.g., syrups and elixirs or the like, wherein a sweetened vehicle is employed. Sustained release compositions can be formulated including those wherein the active compound is protected with differentially degradable coatings, e.g., by microencapsulation, multiple coatings, etc.

Suitable for oral administration are, inter alia, tablets, dragees, capsules, pills, granules, suspensions and solutions. Each unit dose, e.g., each tablespoon of liquid or each tablet, or dragee contains, for example, 5-5000 mg of each active agent.

Solutions for parenteral administration contain, for example, 0.01 - 1% of each active agent in an aqueous or alcoholic solution.

The nitric oxide substrate and/or donor can be administered as an admixture with the progestational agent and any other optional active agent or as a separate unit dosage form, either simultaneously there-with or at different times during the day from each other.

The combination of active agents is preferably administered at least once daily (unless administered in a dosage form which delivers the active agents continuously) and more preferably several times daily, e.g., in 2 to 6 divided doses. The typical dose is about 0.5 to 1000 mg of each active agent, although some less active agents, e.g., L-Arginine, require much higher oral dosages, e.g., 500 to 10,000 mg, and others, e.g., sodium nitroprusside, require lower doses, e.g., 500-2,000 ug/kg/day. Doses for nitroglycerine typically are orally 2.5 mg 2 x daily; sublingually, 0.8 mg 1-4 x dialy; and

transdermally, 0.2-0.4 mg/hr. Since the LD_{50} dosages of most of these active agents is known in the prior art, a lower dosage regimen can be initiated and the dosage increased until a positive effect is achieved or a higher dosage regimen can initially be employed, e.g., in a crisis situation, and the dosages regulated downward as relief from the symptoms is achieved.

In humans, both L-arginine and progesterone (or bioequivalent of another progestin) should be given in a ratio which produces blood plasma levels of about 1-5mMol/ml and 300-1,000 ng/ml (0.9-3μMol/l), respectively. The NO-donor, e.g., sodium nitroprusside, should be given with the progesterone (or bioequivalent of another progestin) in a ratio producing blood plasma levels of about 1-10 μMol/l and 300-1,000 ng/ml (0.9-3μMol/l), respectively.

Brief Description of the Drawings

With reference to the drawings,

FIGURE 1 is a series of strip chart recordings showing the effect of L-arginine on spontaneously contracting uterine strips from rat on day 18 of gestation;

FIGURE 2: Dose-dependent relaxation effects of L-arginine (0.1 mM to 10 mM) on spontaneously contracting uterine strips from rats at different stages of gestation, during delivery adn post partum. The tissues were obtained on days 17-22 (d17, d18, d19 and d22) of gestation, on day 22 (d22 del) during spontaeous delivery (1-3 pups delivered), or on 1 (d1pp) and 2 (d2pp) days postpartum. The duration of complete inhiition of spontaneous uterine contractiosn are dose-dependent. Data are analyzed by repeated measures ANOVA on seven groups. The effects of L-arginine from concentrations of 1 mM are significantly ($P<0.01$) decreased durign spontaeous delivery at term adn postpartum, compared to all other times. Each data point represent mean ± S.E.M.

The total number of strips studied at each time period was 8-16 from 4-6 animals per group.

FIGURE 3: Dose response effects of L-arginine (0.6 mM to 10 mM) on the spontaeous contractility of uterine strips from ovariectomized adult rats. Animals received s.c. injection of 1 ug estradiol - 17b (OVX + E), 2 mg progesterone (OVX + P), estraodiol and progesterone (OVZ + E + P) in sesame oil or oil alone (OVX + Oil) for 3 days prior to contractility measurements. Values are mean ± SEM for 4 strips from each animal from 4 rats per group. Data are analyzed by repeated measures ANOVA on four groups. *P<0.05 OVX + P vs OVX + E.

FIGURE 4: 8-bromo-cGMP dose relaxation-response curves for uterine tissues from rats delivering, spontaeously at term (DEL), preterm with ZK299 (PRETERM DEL) and nondelivering (NONDEL) on day 18 of gestation. Each point represent means ± SEM for 4 strips from each animal from 4 rats per group.

FIGURE 5 is a bar chart which shows the effect on blood pressure of test animals of 50 mg of the hypertensive agent L-NAME, alone or in combination with one or both of L-arginine and progmesterone (R-5020); and

FIGURE 6 is bar chart which shows the effect in the same experiments on pup weights of these compounds.

Discussion of the Drawings

The strip chart recordings of Figure 1 show that the application of L-arginine (1-3 mM) (A, B, E), sodium nitroprusside (5 mM)(C), nitric oxide (0.1 mM) (D) to muscle baths produced substantial relaxations. The effects of L-arginine were reversed by L-NAME (3 mM)(B) and methylene blue (0. mM)(E). These ar etypical recordings of 8-16 strips from 6 animals in each group. Each upstroke from baseline represents a contraction.

The strip chart recording of Figure 1C show that the application of sodium nitroprusside (SNP) caused sustained relaxation in spontaneously contracting uterine strips after a lag period and that tissues in the relax

state were responsive to potassium chloride. Similar recordings of 12 uterine strips from 4 animals were obtained.

The strip chart recording in Figure 1D show the relaxation produced by authentic nitric oxide gas (0.1 mM). Similar recordings were obtained from 8 strips from 4 animals.

The strip chart recordings of Figure 1E show that L-arginine (1 mM) produced relaxation of spontaneously contracting tissues and these effects were repeatable in the same strip (as in Fig. 1A) and that the relaxation effect of L-arginine (1 mM) was abolished by methylene blue (0.1 mM) when added before the application of L-arginine (B).

In the experiments whose results are shown by the graph of Figure 2, the tissues were obtained on days 17-22 (d17, d18, d19 and d22) of gestation, on day 22 (d22 del) during spontaneous delivery (1-3 pups delivered), or on 1 (d1pp) and 2 (d2pp) days postpartum. The duration of complete inhibition of spontaneous uterine contractions are dose-dependent. The effects of L-arginine from concentrations of 1 mM are significantly ($P<0.01$) decreased during spontaneous delivery at term and postpartum, compared to all other times. Each data point represent mean ± S.E.M. The total number of strips studied at each time period was 8-16 from 4-6 animals per group.

In the experiments whose results are shown by the graph of Figure 3, nonpregnant ovariectomized rats received s.c. injection of 1 ug estradiol-17-ß (OVX + E), 2 mg progesterone (OVX + P), estradiol and progesterone (OVX + E + P) in sesame oil or oil alone (OVX + Oil) for 3 days prior to contractility measurements. Values are mean ± SEM for 4 strips from each animal from 4 rats per group. *$P<0.05$ OVX + P vs OVX + E.

The charge of Figure 4 shows 8-bromo-cGMP dose relaxation-response curves for uterine tissues from rats

delivering, spontaeously at term (DEL), preterm with ZK299 (PRETERM DEL) and nondelivering (NONDEL) on day 18 of gestation. Each point represent means ± SEM for 4 strips from each animal from 4 rats per group.

The data in Table 1 below show the effects of L-NAME infusion on blood pressure (mm Hg) in pregnant rats.

TABLE 1

	Blood Pressure (mm Hg)		
Gestation day	CONTROL	L-NAME 25 mg/day	L-NAME 50 mg/day
Day 15	121 ± 3[a]	119 ± 2[a]	123 ± 3[a]
Day 18	119 ± 3[a]	144 ± 4[b]	166 ± 2[c]
Day 22	120 ± 5[a]	146 ± 2[b]	168 ± 3[c]

Means with different superscripts differ significantly ($P<0.05$)

The data in Table 2 below show the delivery and the pups delivered of L-NAME infusion to pregnant rats.

TABLE 2

	CONTROL	L-NAME 25 mg/day	L-NAME 50 mg/day
Day of Delivery	22.3 ± 0.2	22.4 ± 0.2	22.7 ± 0.2
Total # of pups	59	65	56
# of dead pups	2	5	10
Weight of pups	6.32 ± 0.05[a]	5.05 ± 0.08[b]	4.56 ± 0.10[c]
Total # of animals	8	9	10

Means with different superscripts differ significantly ($P<0.05$).

Another experiment using L-NAME-induced" pre-eclampsia" showed that treatment with L-arginine alone partially reduced blood pressure (Figure 5). Similarly, animals treated with L-NAME and R 5020 (promegestone), a progestational agent with no antimineralocorticoid effect

or other antagonistic or agonistic properties, also partinally reduced L-NAME-induced hypertension. As also shown in Figure 5, when the same doses of L-arginine and R 5020 were given simultaneously, their combined effect lowered blood pressure to normal levels.

Additionally, evaluation of fetal weights in the same animals treated as described above, showed intrauterine fetal retardation (decreased weight of pups), typical preeclamptic fetuses (Figure 6). Treatment of the "preeclamptic" groups of animals with either L-arginine alone or R 5020 alone slightly but statistically significant, elevated fetal weights. As also shown in Figure 6, the combined effect of the two compounds administered together significantly elevated fetal weight above that observed with either compound alone, a highly significant advantage to survival of the fetus under these conditions.

It can be concluded from these studies that the combined treatment of L-arginine with a progestational agent whose activity is "pure", like R 5020 provides results which cannot be achieved with either type of drug alone. The studies show that the basis for this effectiveness lies in the ability of the progestional agent to increase the effectiveness of nitric oxide (or L-arginine, the substrate of nitric oxide) to dilate bood vessels and thereby lower blood pressure as well as increase fetalmaternal profusion, thereby increasing fetal weight.

The combined effect of the combination of these agents is surprisingly dramatic and, more importantly, the significant fetal and maternal effects observed with treatment with the combination. Prior medical evidence does not suggest that the combination would provide these advantages, because the basis for them is not the simple combination of two agonistic compounds but instead is the sensitizing of nitric oxide provided by the progestin. The studies clearly indicate that progestins increase the

effector system for nitric oxide (not increase nitric oxide synthesis).

The method of treatment employed in this invention can also be employed for the treatment of hypertension (in both females and males), climacteric disorders (hot flushes, mood swings) in menopausal women, thrombotic disorders, menstrual disorders (dysmenorrhea, functional uterine bleeding), and hemorrhage, etc., following the dosage regime described herein.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the disclosure in any way whatsoever.

The entire disclosure of all applications, patents and publications, cited above and below are hereby incorporated by reference.

Examples

Example 1 - Treatment of Preeclampsia

To a pregnant human female (ca 20-40 years; 60-80 kg) usually in her second half of pregnancy and displaying the symptoms of preeclampsia, including hypertension (above 140 mm systolic and above 90 mm diastolic), edema and protein-uria, administer 0.5 to 20 g of L-arginine and 200 mg of micronized progesterone per os daily in three divided doses until the symptoms are ameliorated. Thereafter, administer 0.5 to 5 mg of L-arginine and 60 mg of progesterone per os daily whenever the diastolic pressure rises above 80 mm; with increasing doses of L-argininine to from 5 to 20 mg daily until remission of the symptoms again occurs.

Example 2 - Treatment of preeclampsia

To a human female comparable to and displaying the same symptoms as the one described in Example 1, administer daily 2 x 2.5 mg of nitroglycerine and 200 mg

of progesterone following the same protocol, until the symptoms are ameliorated.

Example 3 - Treatment of Preterm Labor

To a human female in her sixth month of pregnancy and displaying symptoms of a threatened spontaneous abortion, including blood spotting and periodic uterine spasms, administer daily 17 g of L-arginine and 50 mg of progesterone per os daily in three divided doses until the symptoms are ameliorated. Thereafter, administer 5 g of L-arginine and 50 mg of progesterone per os daily with increasing doses to 20 g of L-arigine daily until remission of the symptoms again occurs.

Example 5 - Treatment of Preterm Labor

To a pregnant human female comparable to and displaying the same symptoms as the one described in Example 3, administer daily 2 x 25 mg of nitroglycerine and up to 180 mg of progesterone, following the same protocol, until the symptoms are ameliorated.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

Patent Claims

1. Use of (a) a progestin and (b) a nitric oxide synthase substrate, a nitric oxide donor or both, optionally, in further combination with one or more of a cyclooxygenase inhibitor, a PGI_2-mimetic, a thromboxane (TXA_2) inhibitor, a compound possessing TXA_2-agonistic and TXA_2-inhibiting properties, a compound possessing TXA_2-antagonistic and PGI_2-mimetic activities, and a TXA_2 antagonist, for manufacture of a medicament for treating at least one of preeclampsia and preterm labor in a pregnant female mammal.

2. Use of claim 1, wherein (a) is used in an amount of which is bioequivalent to 50-300 mg of injected progesterone and (b) in an amount which is effective to raise the blood level of circulating L-arginine to at least about 1 mmole above the normally 2 to 3 mmolar circulating levels.

3. Use of claim 1 or 2, wherein the female mammal is a human suffering from preeclampsia.

4. Use of claim 1 or 2, wherein the female mammal is a human who has exhibited or is a candidate for preterm labor.

5. Use of claim 1 or 2, wherein the female mammal is a human and wherein (b) is a nitric oxide synthase substrate.

6. Use of claim 5, wherein the substrate is L-arginine.

7. Use of claim 1 or 2, wherein the female mammal is a human and wherein (b) is a nitric oxide donor.

8. Use of claim 7, wherein the nitric oxide donor is sodium nitroprusside, nitroglycerin, glyceryltrinitrite, SIN-1, isosorbidmononitrite or isosorbiddinitrite.

9. Use of claim 7, wherein the nitric oxide donor is for oral administration.

10. Use of claim 1 or 2, wherein the female mammal is a human and the nitric oxide substrate or donor is for administration in combination with a cyclooxygenase inhibitor.

11. Use of claim 10, wherein the inhibitor is aspirin.

12. Use of claim 1 or 2, wherein the female mammal is a human and the nitric oxide substrate or donor is for administration in combination with a PGI_2-mimetic.

13. Use of claim 12, wherein the PGI_2-mimetic is iloprost or cicaprost.

14. Use of claim 1 or 2, wherein the female mammal is a human and the progestin is progesterone

15. A pharmaceutical composition comprising an admixture of (a) a progestin and (b) a nitric oxide synthesis substrate, a nitric oxide donor or both, and optionally, also at least one of a cyclooxygenase inhibitor, a PGI_2-mimetic, a thromboxane (TXA_2) inhibitor, a PGI_2-mimetic, a thromboxane (TXA_2) inhibitor, a compound possessing TXA_2-agonistic and TXA_2-inhibiting properties, a compound possessing TXA_2-antagonistic and PGl_2-memetic activities, and a TXA_2 antagonist, in amounts effective to ameliorate the symptoms of

preeclampsia, toxemia or preterm labor in a pregnant female mammal when administered thereto in an amount effective provide an amount of the progestin bioequivalent to 50-300 mg. of injected progesterone and an amount of the nitric oxide synthase substrate, nitric oxide donor or both effective to raise the blood level of circulating L-arginine to at least about 1 mmole above the normally 2 to 3 mmolar circulating levels or raise the nitric oxide donor levels to about 1 to 1000 nmolar.

16. The composition according to claim 15, wherein (b) is a nitric oxide synthesis substrate.

17. The composition according to claim 16, wherein the nitric oxide synthesis substrate is L-arginine.

18. The composition according to claim 15, wherein (b) is a nitric oxide donor.

19. The composition according to claim 18, wherein the nitric oxide donor is sodium nitroprusside, nitroglycerin, glyceryltrinitrie, SIN-1, isosorbidmononitrite or isosorbiddinitrite.

20. The composition according to claim 18, which comprises a cyclooxygenase inhibitor.

21. The composition according to claim 18, which comprises a PGI_2-mimetic.

22. The composition according to claim 18, which comprises a thromboxane inhibitor.

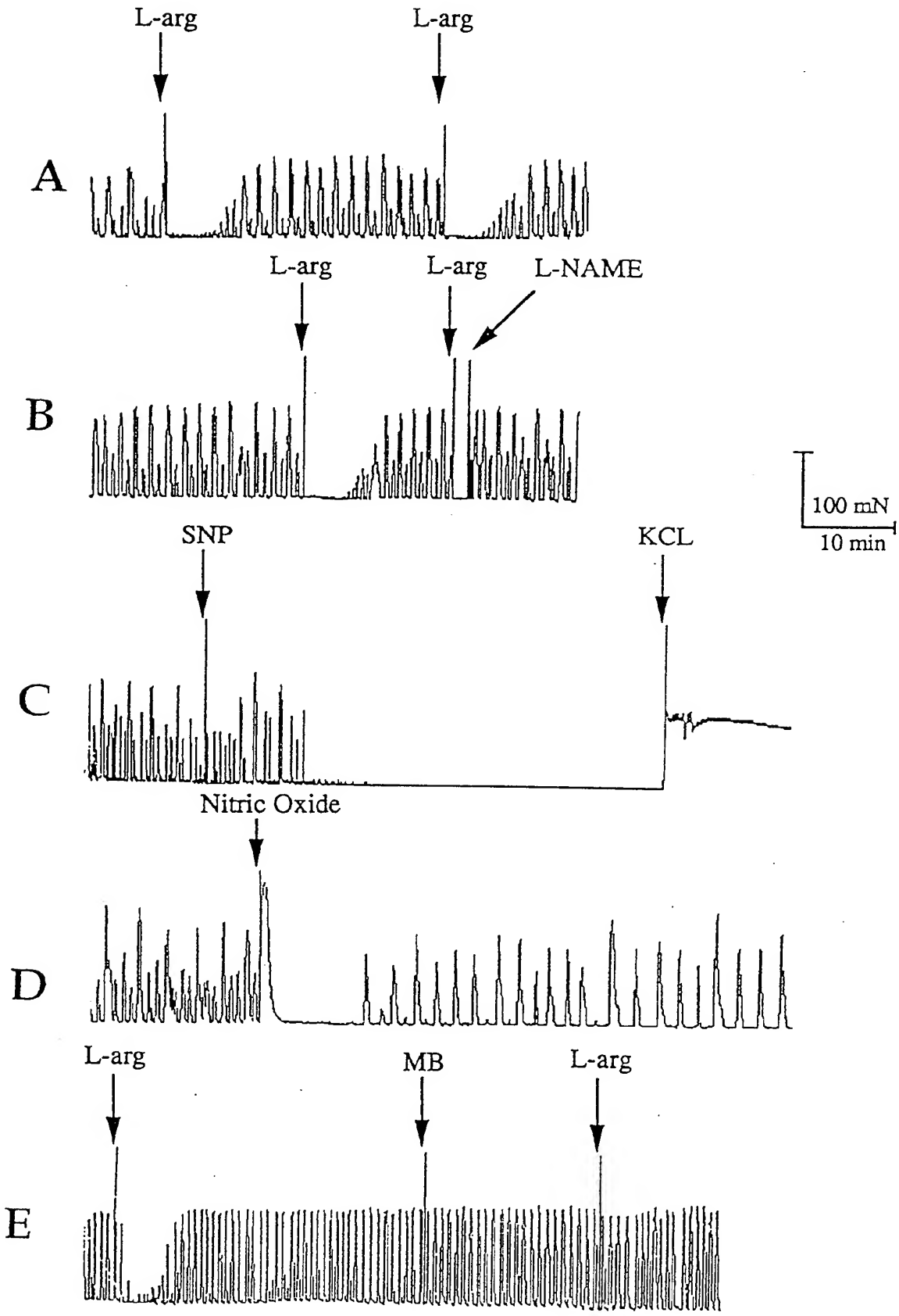
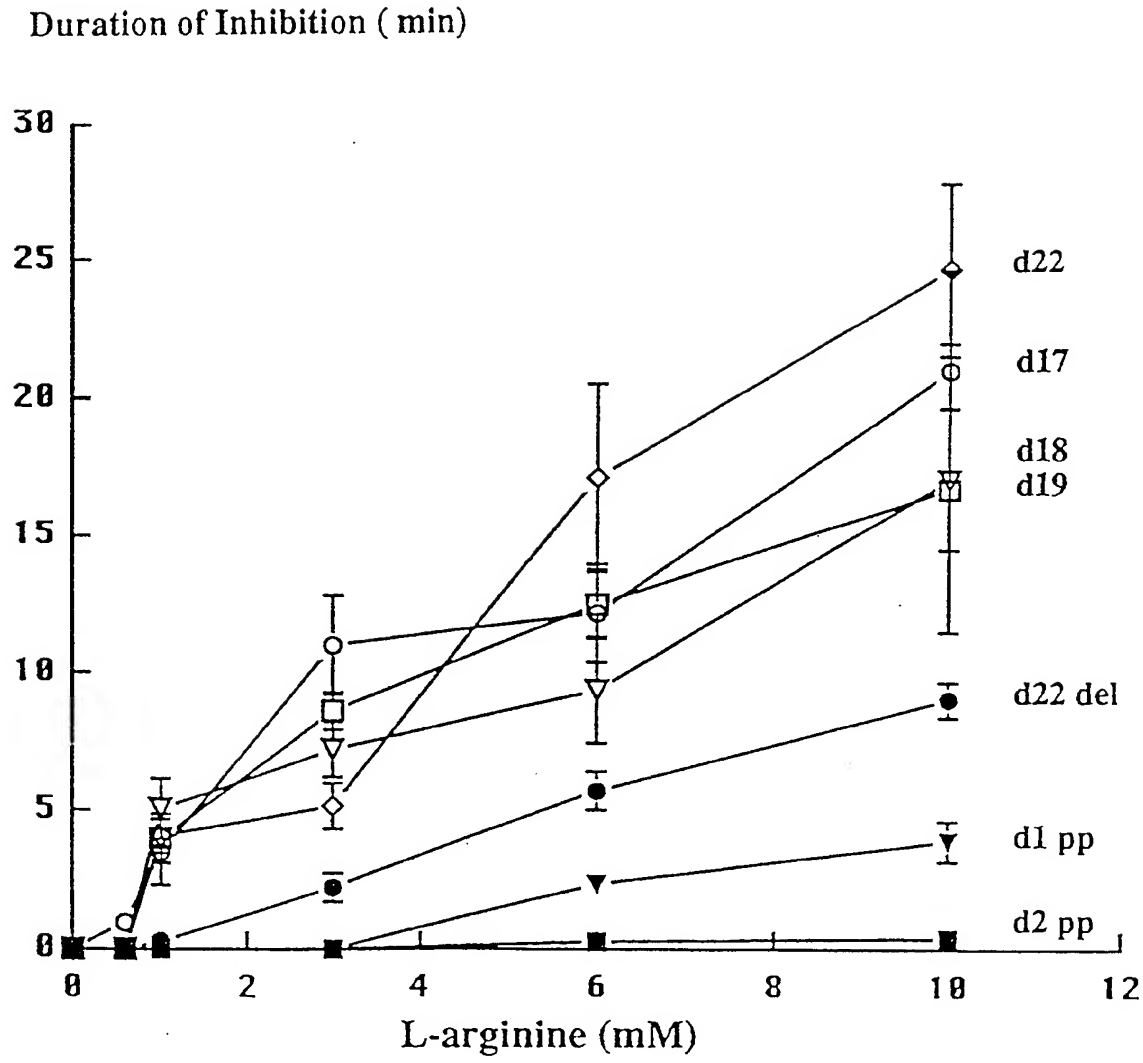

L-arg
L-arg
A
L-arg
L-arg
L-NAME
B
100 mN
10 min
SNP
KCL
C
Nitric Oxide
D
L-arg
MB
L-arg
E

Fig 1


Duration of Inhibition (min)
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25
20
15
10
5
0
0
2
4
6
8
10
12
L-arginine (mM)
d22
d17
d18
d19
d22 del
d1 pp
d2 pp

Fig. 2

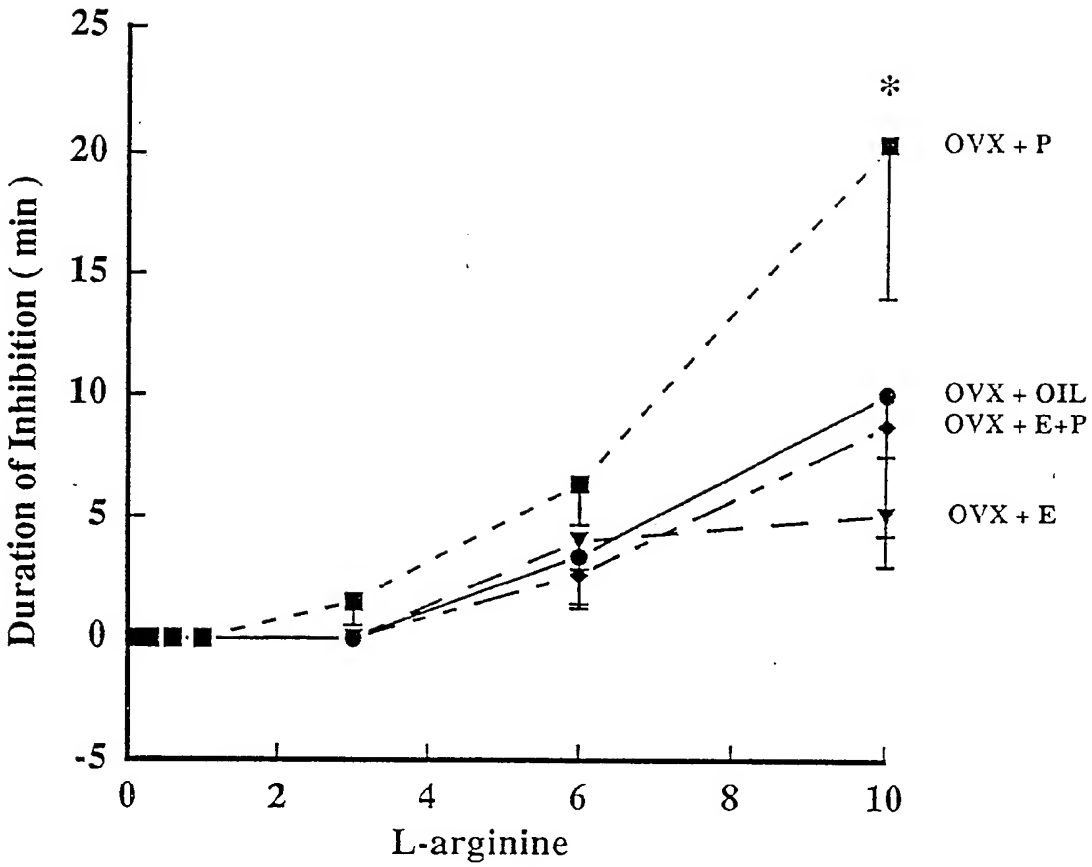
25
20
15
10
5
0
-5
Duration of Inhibition (min)
0
2
4
6
8
10
L-arginine
*
OVX + P
OVX + OIL
OVX + E+P
OVX + E

Fig. 3

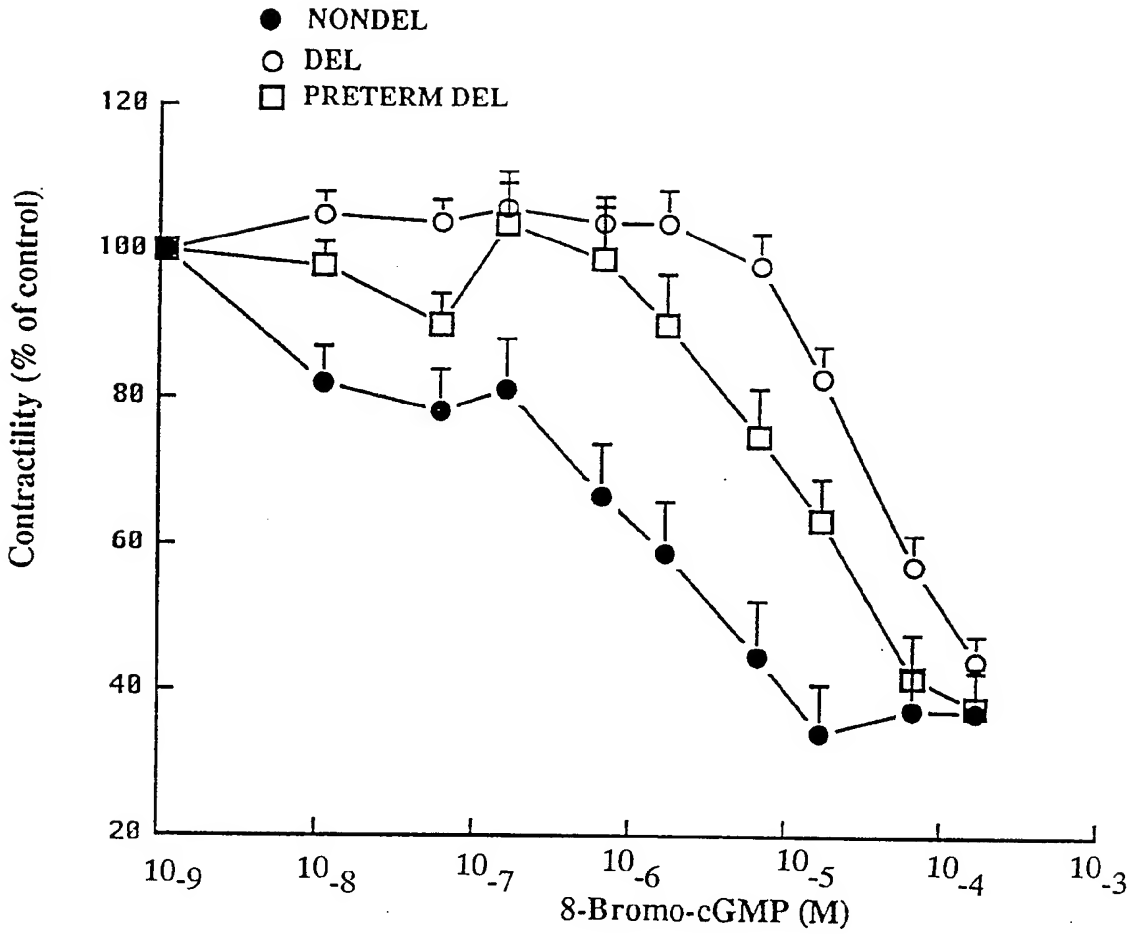
NONDEL
DEL
PRETERM DEL
Contractility (% of control)
120
100
80
60
40
20
10-9
10-8
10-7
10-6
10-5
10-4
10-3
8-Bromo-cGMP (M)

Fig. 4

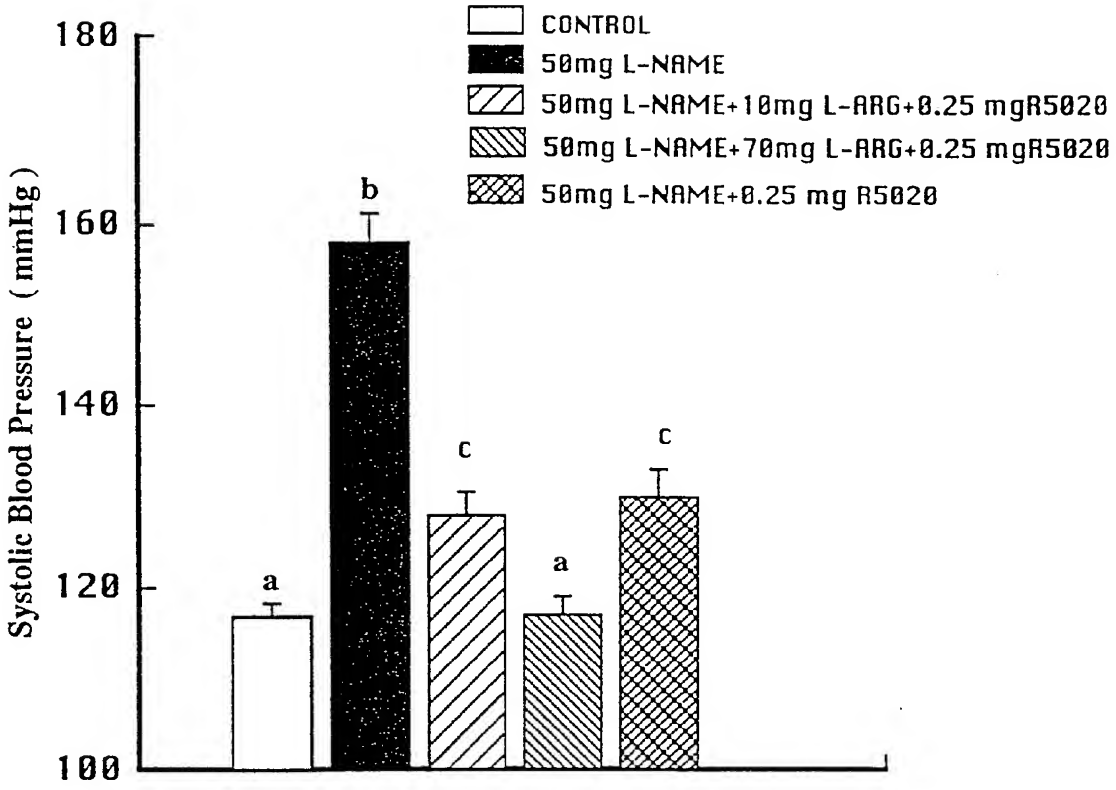
CONTROL
50mg L-NAME
50mg L-NAME+10mg L-ARG+0.25 mgR5020
50mg L-NAME+70mg L-ARG+0.25 mgR5020
50mg L-NAME+0.25 mg R5020
Systolic Blood Pressure (mmHg)
180
160
140
120
100
a
b
c
a
c

Fig. 5

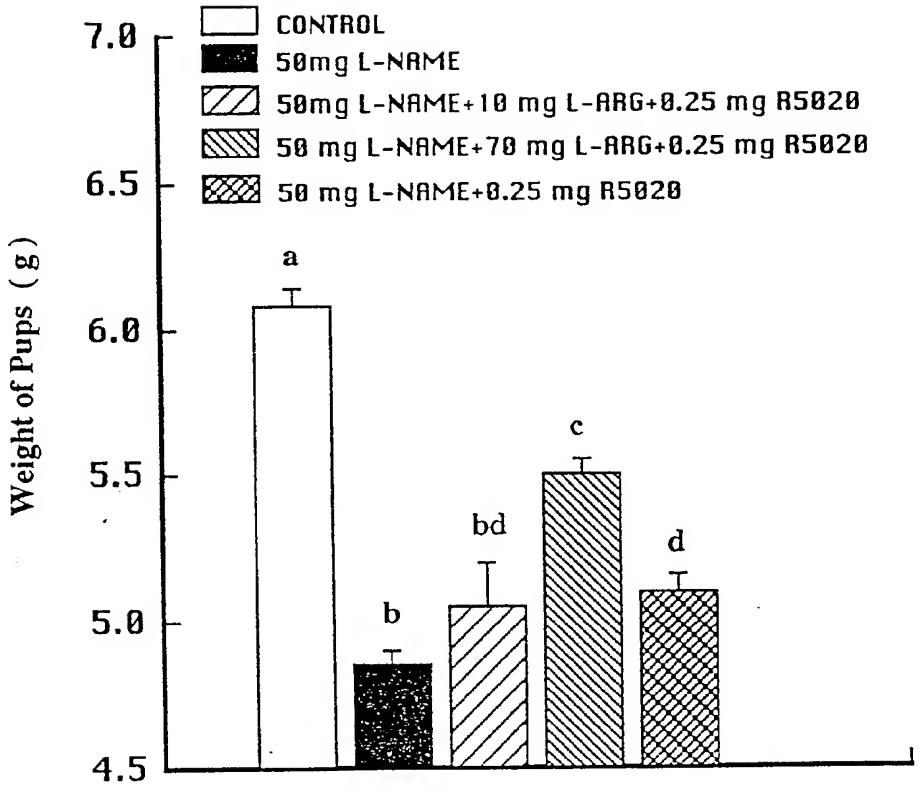
CONTROL
50mg L-NAME
50mg L-NAME+10 mg L-ARG+0.25 mg R5020
50 mg L-NAME+70 mg L-ARG+0.25 mg R5020
50 mg L-NAME+0.25 mg R5020
Weight of Pups (g)
7.0
6.5
6.0
5.5
5.0
4.5
a
b
bd
c
d

Fig. 6

INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/EP 94/02368

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/57 //(A61K31/57,31:34,31:26,31:21,31:195)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 441 119 (LEVERE, RICHARD D. ET AL.) 14 August 1991 cited in the application see abstract -----	1-15

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 November 1994	30. 11. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Leherte, C

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 94/ 02368

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-15
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
FOR FURTHER INFORMATION SEE ANNEX

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

CONTINUATION OF BOX I.2

In view of the large number of compounds which are defined by the wording of the claims, the search has been performed on the general idea and compounds metioned in the examples of the description (PCT art.6; Guidelines, Part B, chapt. II.7 last sentence and chapt. III.3.7.)

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Interna ...l Application No
PCT/EP 94/02368

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0441119	14-08-91	US-A- 5217997	08-06-93